IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Laursen, I et al.
Serial no.	:	09/902,174
Filed	:	July 10, 2001
For	:	Process for producing immunoglobulins for intravenous administration and other immunoglobulin products
Examiner	:	D. Saunders
Art unit	:	1644

Declaration of Inga Laursen

1. I, Inga Laursen, Charlottenlundvej 9, 1. tv. 2900 Hellerup, Denmark, in my capacity as Manager, PhD at Statens Serum Institut do state and declare as follows:

2. I am among the named inventors of the above-captioned patent application. I believe that I am a person skilled in the art to which the above-captioned application pertains.

3. I have performed the following calculations and experiment in order to further substantiate the difference between the immunoglobulin for intravenous administration of the present application and the immunoglobulin product disclosed in United States Patent 4,880,913 issued to Doleschel et al.

4. Calculation of residual PEG content in the immunoglobulin product disclosed by Doleschel et al.:

Doleschel et al. precipitate labile and aggregated IgG molecules by addition of PEG to a final concentration of 10% to 25% w/w, such as 10% to 15% w/w PEG according to the claims. PEG is removed by ultrafiltration according to the claims, however, neither the concentration of residual PEG nor the volumes used for ultrafiltration is mentioned.

In the examples where five times the amount of buffer is used, the concentration of PEG will be bisected for each volume buffer used for ultrafiltration. In a best cast scenario the lowest PEG amount is added: 10% w/w $\geq$ 100 mg PEG/ml will be the start conditions before ultrafiltration.

PEG concentration	**% weight/weight**	**mg/ml**
start	10	100.00
1 volume buffer exchange	5.00	50.00
2 volumes buffer exchange	2.500	25.00
3 volumes buffer exchange	1.250	12.50
4 volumes buffer exchange	0.6250	6.250
5 volumes buffer exchange	**0.3125**	**3.125**
6 volumes buffer exchange	0.1563	1.563
7 volumes buffer exchange	0.0781	0.781
8 volumes buffer exchange	0.0391	0.391
9 volumes buffer exchange	0.0195	0.195

10 volumes buffer exchange	0.0098	0.098
11 volumes buffer exchange	0.0049	0.049
12 volumes buffer exchange	**0.0024**	**0.024**
13 volumes buffer exchange	0.0012	0.012

Conclusion:
As shown above, after 5 volumes of buffer exchange the residual PEG concentration in the product disclosed by Doleschel et al. will be at least 3.1 mg/ml. The final PEG level in the IVIG product of the present invention, on the other hand, is 0.02 mg/ml according to Example 2. To reach such a low PEG level in the product of Doleschel would take at least 12 -13 volumes of buffer for ultrafiltration. Accordingly, the two products are clearly distinguishable in terms of their PEG content.

5. Sensitivity of the Outcherloney technique:

With the aim to study the sensitivity of a double diffusion-in-gel technique for the IgA rabbit-anti-IgA system a dilution row of a commercial IgA standard was prepared an analysed against a series of rabbit-anti-IgA concentrations by the Ouchterlony assay.

1% Agarose gel
Agarose: BF-010-36
Buffer: Tris/Tricin 24.09.03 (diluted 5-fold)
Boiled agarose used for casting 6.5x8 cm gel-bond plates:

Dilution row of IgA
IgA standard: DAKO, code X0908, lot 102, with IgA concentration: 2.27 mg/ml
Dilute 2-fold in the buffer used for agarose gel to following concentrations:
50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.3 µg/ml, 3.1 µg/ml, 1.6 µg/ml

Dilution of rabbit-anti-IgA antibody
Rabbit-anti-IgA antibody: DAKO, code A0092, lot 069
Antibody concentration: 7.5 mg/ml
Dilute 2-fold in the buffer used for agarose gel to following concentrations:
100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.3 µg/ml, 3.1 µg/ml, 1.6 µg/ml.

Seven Ouchterlony assay gels are set up, with the seven different antibody dilutions in the central wells, and the 6 IgA dilutions in the 6 surrounding well on each gel. Wells are loaded with 10 µl of all diluted samples.
The gels are incubated for 3 days in a humid box at room temperature.
The gels are dried and stained with Coomassie brilliant blue for visualising of precipitation lines.

Results
After staining no specific precipitation arcs or lines were observed on any of the seven gels. This indicates that the sensitivity of the double diffusion technique performed as described above is not high enough to reveal visual immuno-precipitates and by this determine the IgA concentrations analysed.

6. I further declare that all statements made herein of my own knowledge are true and further that the statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001

of Title 18 of the United States Code and that such wilful false statements may jeopardise the validity of the application or any patent issued thereon.

Dated: November 25 2003 Signature: Inga Laursen

Inga Laursen